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SEMIOCHEMISTRY OF THE GOLDENEYED LACEWING *Chrysopa oculata*: ATTRACTION OF MALES TO A MALE-PRODUCED PHEROMONE[1]

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Abstract—Gas chromatographic-electroantennographic detection (GC-EAD) experiments showed that antennae of males and females of the goldeneyed lacewing, *Chrysopa oculata* Say (*Co.* = *Chrysopa*), consistently responded to four compounds extracted from the abdominal cuticle of males: nonanal, nonanol, nonanoic acid, and (1*R**,2*S**,5*R**,8*R**)-iridodial. These compounds were not detected from abdominal cuticle of females. Thoracic extracts of both sexes contained antennal-stimulatory 1-tridecene and EAD-inactive skatole. *Chrysopa oculata* adults were most sensitive to (1*R*,2*S*,5*R*,8*R*)-iridodial standard at an EAD-response threshold between 0.1 and 1 pg, which was 10–100 times lower than thresholds for nonanal and nonanoic acid, and up to 10,000 times lower than thresholds for other compounds tested. A similar EAD response pattern was also found in another *Chrysopa* sp. (*Co. quadripunctata* Burmeister). In field-trapping experiments, (1*R*,2*S*,5*R*,8*R*)-iridodial was the only male-specific compound that attracted *Co. oculata* males. Males also were weakly attracted to (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (an aphid sex pheromone component), probably due to the 5% (1*R*,2*S*,5*R*,8*R*)-iridodial present in the synthetic sample as an impurity. A herbivore-induced plant volatile, methyl salicylate, increased attraction of males to (1*R*,2*S*,5*R*,8*R*)-iridodial, whereas 1-tridecene was antagonistic. No females were caught in the entire study. Scanning electron micrographs revealed numerous male-specific, elliptical epidermal glands on the 3rd–8th abdominal sternites of *Co. oculata*, which are

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likely the pheromone glands. Another lacewing species, *Chrysoperla rufilabris* (Burmeister) (*Cl.* = *Chrysoperla*), did not produce male-specific volatiles or possess the type of gland presumed to produce pheromone in *Co. oculata* males, but (*Z*)-4-tridecene was identified as a major antennal-stimulatory compound from thoracic extracts of both sexes of *Cl. rufilabris*. Thus, (1*R*,2*S*,5*R*,8*R*)-iridodial (or its enantiomer) is now identified as a male-produced male aggregation pheromone for *Co. oculata*, the first pheromone identified for lacewings.

Key Words—Neuroptera, Chrysopidae, attractant, pheromone, synomone, kairomone, methyl salicylate, iridodial, tridecene, GC-EAD.

INTRODUCTION

Lacewings, especially green lacewings (Chrysopidae), are some of the most common predators of aphids and other soft-bodied insects (New, 1975; Tauber et al., 2000). Because of their commercial availability and resistance to insecticides, chrysopids are among the most commonly released predators for augmentative biological control (Ridgway and Murphy, 1984; Tulisalo, 1984; Aldrich, 1999) albeit with differing degrees of success (Rosenheim, 1998). Whereas green lacewings are increasingly being released for biocontrol, methods are still needed to retain the predators near augmentation sites and/or to attract wild populations to target areas (Baker et al., 2003).

Recent studies on semiochemicals involved in lacewing-related systems suggest that interspecific signals might improve biocontrol using lacewings. For example, various lacewings are attracted to host plant volatiles (Flint et al., 1979; Hagen, 1986; Zhu et al., 1999; Hooper et al., 2002), herbivore-induced plant volatiles (James, 2003), and sex pheromones of scale insects (Mendel et al., 2003), and aphids (Boo et al., 1998, 1999; Hooper et al., 2002). In particular, some lacewings are attracted to certain isomers of nepetalactone and nepetalactol, which are components of aphid pheromones and are also found in the catnip plant (Lamiaceae: *Nepeta cataria*). However, the explanation that this attraction is a means to find aphid prey (Boo et al., 1998) has been questioned because only male lacewings are attracted, and aphids do not produce pheromone until late summer or autumn (Hooper et al., 2002). Hooper et al. (2002) suggested that aphid pheromone compounds, or analogous structures, may play a pheromonal role for lacewings, and that their similarity to aphid sex pheromones may be simply incidental.

Intraspecific chemical signals may have even more potential for managing lacewings, but pheromones of chrysopids have yet to be identified. Therefore, the present investigation was initiated to (1) search for pheromones of *Chrysopa oculata* Say (*Co.* = *Chrysopa*) and *Chrysoperla rufilabris* (Burmeister) (*Cl.* = *Chrysoperla*), the most common lacewings in the eastern United States, (2) compare the attraction of suspected pheromones and other known lacewing semiochemicals, and (3) test the potential antagonistic effect of allomone compounds on attraction.

METHODS AND MATERIALS

Adult Insects and Preparation of Extracts. Lacewings for chemical and GC-EAD analyses were collected in traps in late May 2003 during a preliminary field test (see below), and by sweep netting during June–August, 2003. Most *Co. oculata* adults were collected from an alfalfa field during daylight hours on the North Farm of the Beltsville Agricultural Research Center (BARC), Prince George's County, Maryland, whereas *Cl. rufilabris* adults were captured near a light in the backyard of a house in College Park, Maryland, between 21:30 and 23:30 hr. Adults of both species were dissected within 6–20 hr of capture for electrophysiological and chemical analyses. For extraction, *Co. oculata* adults were anesthetized with CO_2, eviscerated under tap water, the body parts were cut as follows, dried with tissue paper, and extracted individually in 50 μl of methyl tert-butyl ether: abdominal cuticle (segments 1–8), abdominal tip (last segment), and thorax. Thoracic and abdominal extracts of *Cl. rufilabris* were made in the same manner as for *Co. oculata*. All the extracts were kept at –20°C until GC-EAD/MS analyses. A few *Chrysopa quadripunctata* Burmeister males were collected from the sticky traps and used for GC-EAD analysis.

Gas Chromatography-Electroantennogram Detector (GC-EAD) Analysis. Lacewing extracts and chemical standards were analyzed in splitless mode using an HP 6890 GC equipped with a DB-WaxETR column (0.25 μm film thickness, 30 m × 0.25 mm ID; J & W Scientific, Folsom, CA), and a 1:1 effluent splitter that allowed simultaneous flame ionization detection (FID) and EAD of the separated volatile compounds. Helium was used as the carrier gas, and the injector temperature was 220°C. The column temperature was 50°C/2 min, rising to 240°C at 10°C/min, then held for 10 min. The outlet for the EAD was held in a humidified air stream flowing at 0.5 m/sec over an antennal preparation. EAD recordings were made using silver wire-glass capillary electrodes filled with Beadle-Ephrussi Ringer (Zhang et al., 2000) on freshly cut antennae of both sexes. The antennal signals were stored and analyzed on a PC equipped with a serial IDAC interface box and the program EAD ver. 2.5 (Syntech, Hilversum, The Netherlands). In addition, GC-EAD dose-responses to a synthetic mixture containing five *Co. oculata*-produced compounds [1-tridecene, nonanal, nonanol, nonanoic acid, and (1*R*,2*S*,5*R*,8*R*)-iridodial]; three compounds associated with aphid prey: (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone (sex pheromone components), plus the nonpheromone compound, (4a*S*,7S,7a*S*)-nepetalactone; and one herbivore-induced plant volatile (methyl salicylate) were tested with both sexes of *Co. oculata*. Test compounds were dissolved in hexane (0.001, 0.01, 0.1, 1, 10, 100 ng/μl), and (*E*)-2-hexenyl butyrate (100 ng/μl) was used as positive control. For each GC-EAD run, ca. 2 μl of a test mixture plus 2 μl of the control were combined in the same syringe and then injected. Thus, after a 50:50 split at the end of the GC column, ca. 100 ng of (*E*)-2-hexenyl butyrate and 0.001 to 100 ng of each test compound (depending on the dosage) passed over the antennal

preparation. The EAD responses were then normalized as the percentage response to the control. The same synthetic mixture (100 ng/μl for each compound) was also tested on antennae of *Co. quadripunctata* (males only) and *Cl. rufilabris* (both sexes).

Chemical Standards (Figure 1). 1-Tridecene (97%), 1-nonanol (97%), and skatole (98%) were obtained from Aldrich Chemical (Milwaukee, WI), nonanal (99%) and (*E*)-2-hexenyl butyrate (98%) from Bedoukian Research (Danbury, CT), nonanoic acid (98%) from Emery Industries (Cincinnati, OH), and methyl salicylate (98%) from Fisher Scientific (Fair Lawn, NJ). (*Z*)-4-Tridecene was synthesized by condensing the ylide of *n*-butyltriphenyl phosphonium bromide with octanal following standard Wittig reaction procedure. The product was fractionally distilled to yield 98% 4-tridecenes (*Z*:*E* 93:7).

(4a*S*,7*S*,7a*R*)-Nepetalactone and (4a*S*,7*S*,7a*S*)-nepetalactone are often referred to as *Z*,*E*- and *E*,*Z*-nepetalactone, respectively, in the literature (e.g.,

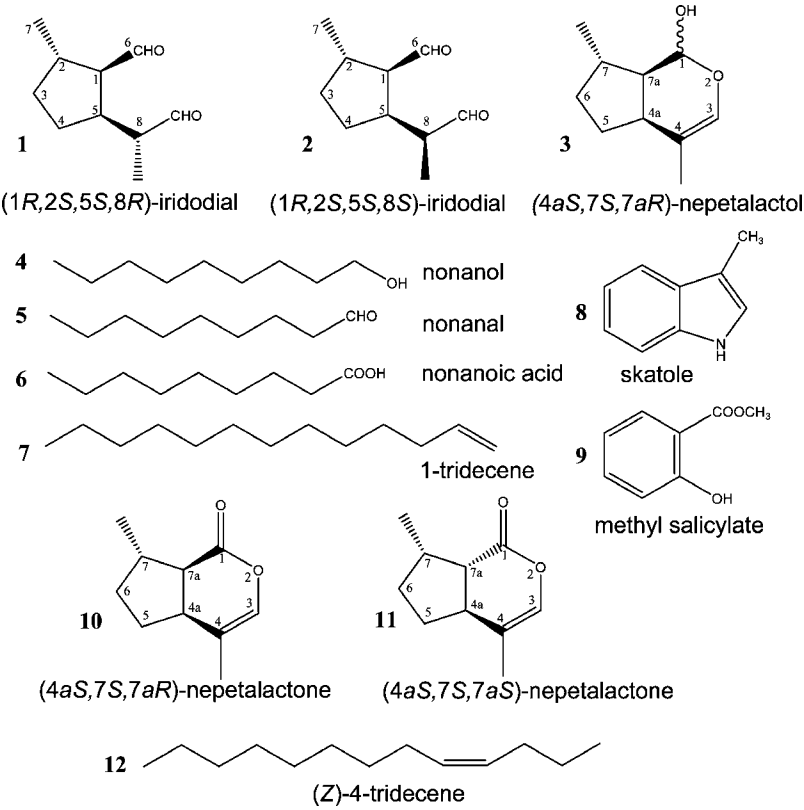

FIG. 1. Compounds identified and/or tested.

Hooper et al., 2002). (4a*S*,7*S*,7a*R*)-Nepetalactone (98%) and (4a*S*,7*S*,7a*S*)-nepetalactone (96%) used for the preliminary test in the spring of 2003 were isolated from commercial catnip oil (Health and Herbs, Philomath, OR) by silica gel flash chromatography using 5% ethyl acetate in hexane (Chauhan et al., 2004). (4a*S*,7*S*,7a*R*)-Nepetalactone (98%) used for later 2003 tests was prepared from commercial catnip oil by a pH-sensitive chemical separation technique (patent pending). (4a*S*,7*S*,7a*R*)-Nepetalactol [predominantly the 1*R*-isomer (Hooper et al., 2002), with ca. 5% impurity of iridodial isomers] was prepared by $NaBH_4$ reduction of (4a*S*,7*S*,7a*R*)-nepetalactone as previously described (Hooper et al., 2002). (1*R*,2*S*,5*R*,8*R*)-Iridodial [80%, with 20% (1*R*,2*S*,5*R*,8*S*)-iridodial as an impurity] was synthesized as described by Chauhan et al. (2004).

GC and GC-Mass Spectrometry (GC-MS) Analyses. Retention time (RT) comparisons, in addition to those provided by GC-EAD, and coinjections were performed in split mode using an HP 6890 GC equipped with a DB-5 column (0.25 μm film thickness, 30 m $\times$ 0.32 mm ID; J & W Scientific, Folsom, CA). Hydrogen was used as the carrier gas, programming from 50°C/2 min, to 250°C at 10°C/min, then held for 10 min. GC-MS was performed with an HP 6890 GC coupled to an HP 5973 mass selective detector using a DB-WaxETR column as above (except 60-m-long column), programmed at 50°C/2 min, rising to 230°C at 15°C/min, then held for 15 min. Male-produced compounds from *Co. oculata* were quantified by the internal standard method using (*E*)-2-hexenyl butyrate. Dimethyldisulfide (DMDS) derivatization (Buser et al., 1983) followed by GC-MS analysis was used to determine double bond positions.

Scanning Electron Microscopy. Live lacewings (males and females) of both species (*Co. oculata* and *Cl. rufilabris*) were anesthetized with CO_2, mounted on copper specimen holders (16 $\times$ 29 $\times$ 1.5 mm thick) with cryoadhesive, and immersed in liquid N_2. The frozen specimens were transferred to an Oxford CT1500 HF cryo preparation system, and examined using a low temperature scanning electron microscope (LTSEM; Hitachi S-4100) operated at 2.0 kV (see Erbe et al., 2003, for details). Micrographs were recorded on Polaroid Type 55 P/N film.

Field Trapping. A preliminary experiment was conducted from March 26 through June 5, 2003 to test potential repellent effects of nepetalactone isomers against the spined soldier bug (Heteroptera: Pentatomidae: *Podisus maculiventris* Say) and its parasitoids. In this test, clear plastic funnel traps baited with synthetic pheromone of the spined soldier bug were deployed as previously described (Aldrich et al., 1984) with or without nepetalactone isomers added. There were eight replicates per treatment plus unbaited control traps, and the nepetalactone treatments consisted of 15 mg of either (4a*S*,7*S*,7a*R*)-nepetalactone or (4a*S*,7*S*,7a*S*)-nepetalactone in100 μl CH_2Cl_2 applied to a separate rubber septum placed in the trap bottom.

Field-trapping experiments designed for *Co. oculata* were carried out in a meadow at BARC-East (experiment 1), and an alfalfa field on the BARC North Farm (experiments 2–3), from July through the beginning of September 2003, using Jackson delta sticky traps (Agrisense, Fresno, CA) baited with 5 mg of test compound(s) in 50 μl of heptane applied to grey rubber septa (5 mm sleeve-type; The West Co., Lititz, PA). Rubber septa were replaced twice per week. Traps were hung 0.8–1.0 m above ground on metal stakes ca. 8 m apart, with ca. 10 m between trap lines. For each experiment, 2–5 sets of traps (a set = one line of traps) were deployed with their initial trap positions being randomized within a set. The trap positions were then systematically rotated within a set after each replicate based on a procedure of Latin-square design (Byers, 1991) so that traps appeared at least once per location. To minimize positional effects, lacewing collections and trap rotations were carried out when $\geq$2 lacewings were caught in the best trap. Each replicate lasted 1–2 d, depending on flight activity. The sticky inserts were taken to the laboratory for recording lacewing species, gender, and numbers.

Experiment 1 (July 18 to August 1, 2003) was conducted with 5 sets of traps to determine behavioral activity of male-specific compounds that stimulated antennae in comparison with (4a*S*,7*S*,7a*R*)-nepetalactone and (4a*S*,7*S*,7a*R*)-nepetalactol. Experiment 2 (August 5 to September 2, 2003) consisted of two sets of traps to test potential activity of the four male-specific compounds, (1*R*,2*S*,5*R*,8*R*)-iridodial, nonanal, nonanol, and nonanoic acid, in a four-way factorial design, i.e., four individual components and all their possible binary, ternary, and quaternary blends (in the same ratio). Experiment 3 (August 21 to September 1, 2003) tested, in four sets of traps, the potential interaction between (1*R*,2*S*,5*R*,8*R*)-iridodial and methyl salicylate, and 1-tridecene. (4a*S*,7*S*,7a*R*)-Nepetalactone and a mixture of nonanal, nonanol, and nonanoic acid (at 1:1:1; on one dispenser) were also included for comparison.

Statistical Analysis. Because of heterogeneity of variances among treatments, trap catch data (number of lacewings caught/trap/replicate) were analyzed using the nonparametric Kruskal-Wallis ANOVA on rank test, followed by the Student-Newman-Keuls all pair-wise comparison to separate means (Zar, 1984).

RESULTS

GC-EAD and Chemical Identifications. In general, antennal preparations of all three lacewing species tested remained active for at least 2 hr, which allowed time for 3–4 GC-EAD runs per preparation. The response of *Co. oculata* antennae toward synthetic (4a*S*,7*S*,7a*R*)-nepetalactol (**3**) is shown in Figure 2A; the antennae of both males and females (not shown) were much more responsive to two impurities in this synthetic standard (**1** and **2**; RT = 15.15 and 15.40 min, respectively) than to nepetalactol (**3**). The GC trace of the *Co. oculata* 1st–8th abdominal

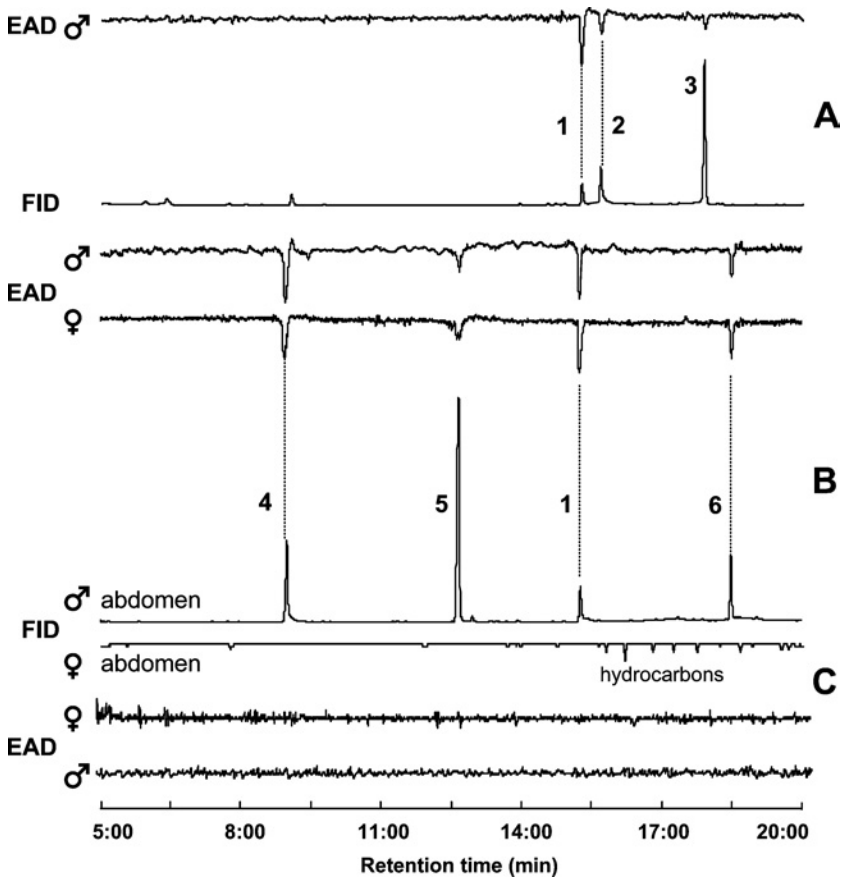

FIG. 2. GC-EAD responses of *Chrysopa oculata* antennae to (A) synthetic (4a*S*,7*S*,7a*R*)-nepetalactol (**3**, see Figure 1), and to cuticular extracts of the 1st–8th abdominal segments of conspecific males (B) and females (C).

extract from males revealed four main peaks (Figure 2B), none of which was observed in the corresponding extract from conspecific females (Figure 2C). The major component of the *Co. oculata* male abdominal extract (**5**) elicited the weakest EAD response of the four major components in this extract. The other three main components elicited strong responses from the antennae of both sexes, with the response to **1** consistently being strongest and the RT of **1** matching that of the first EAD-active impurity in the synthetic (4a*S*,7*S*,7a*R*)-nepetalactol standard (Figure 2B). In contrast, no EAD-active compounds were detected from extracts of the 1st–8th abdominal cuticle of *Co. oculata* females (Figure 2C) or abdominal tip extracts (not shown).

GC-MS analyses revealed that the earlier eluting EAD-active impurity in the nepetalactol standard and the corresponding *Co. oculata* male-specific component (Figure 2A and B, respectively) exhibited identical MS data: *m/z* (%) 67 (65), 81 (100), 109 (35), 135 (18), 150 (8), 153 (4), 168 (3, M^+). Nepetalactol is a monoterpene enol-lactol that exists in equilibrium with iridodials depending on physiochemical conditions (El-Naggar and Beal, 1980). The mass spectra of these natural products matched the published spectra for iridodial (Cavill et al., 1976). Because iridodial standards **1** and **2** were derived from (*Z*,*E*)-nepetalactone **10**, the absolute configuration remains intact for the (7a)-, (7)-, and (4a)-positions of origin (Dawson et al., 1996). The absolute configuration of the newly generated chiral center at C-8 in iridodials **1** and **2** was established by nuclear magnetic resonance spectroscopy (Chauhan et al., 2004). In iridodial **1**, the coupling constant between H5 and H8 ($J = 13.2$ Hz) indicated a *threo* or *trans* configuration (8*R*), whereas in iridodial **2**, $J = 11.3$ Hz between H5 and H8 confirmed an *erythro* or *cis* configuration (8*S*). The relative stereochemistry of lacewing-derived iridodial was identified by coinjection of natural extract with synthetic standards of (1*R*,2*S*,5*R*,8*R*)- and (1*R*,2*S*,5*R*,8*S*)-iridodial. The lacewing-derived material coeluted with (1*R*,2*S*,5*R*,8*R*)- iridodial (RT = 13.30 min), confirming the structure of the *Co. oculata* male-specific product as (1*R*,2*S*,5*R*,8*R*)-iridodial (**1**) or the enantiomer, (1*S*,2*R*,5*S*,8*S*)-iridodial. Male-specific compounds **4**, **5**, and **6** from *Co. oculata* were identified as nonanal, nonanol, and nonanoic acid, respectively, by matching GC and GC-MS data to that of the known standards. The *Co. oculata* male-specific compounds were quantitated as follows (mean μg/male $\pm$ SE; $N = 9$): nonanal (0.91 ± 0.3), nonanol (2.2 ± 0.5), (1R^*,2S^*,5R^*,8R^*)-iridodial (0.26 ± 0.1), and nonanoic acid (0.35 ± 0.1). The coefficients of variation (CV = SD $\times$ 100/mean) in relative amounts (= proportions) of the individual male-specific components were 37% (**4**), 20% (**5**), 112% (**1**), and 56% (**6**).

GC-EAD analyses of thoracic extracts of *Co. oculata* indicated the presence of two main compounds (**7** and **8**), but only the former component was EAD-active (Figure 3). These compounds were identified as 1-tridecene (**7**) and skatole (**8**) by GC and GC-MS comparisons with standards, confirming their earlier identification from this species (Blum et al., 1973).

GC-EAD analyses with *Co. oculata* antennae using synthetic mixtures showed that nonanal, nonanoic acid, and (1*R*,2*S*,5*R*,8*R*)-iridodial elicited much higher EAD responses than did nonanol (**5**), 1-tridecene (**7**), (4a*S*,7*S*,7a*R*)-nepetalactone (**10**), (4a*S*,7*S*,7a*S*)-nepetalactone (**11**), 4a*S*,7*S*,7a*R*-nepetalactol (**3**), or methyl salicylate (**9**) (Figure 4). There were no significant differences in antennal responses between males and females in these tests. GC-EAD dose–response analyses (doses ranging from 1 pg up to 100 ng for each tested compound) showed a significant dose-effect for all the compounds tested. The average EAD response for 100 ng of (*E*)-2-hexenyl butyrate, the active control, was ca. 0.2 mV ($N = 53$). Antennae of both sexes of *Co. oculata* were most

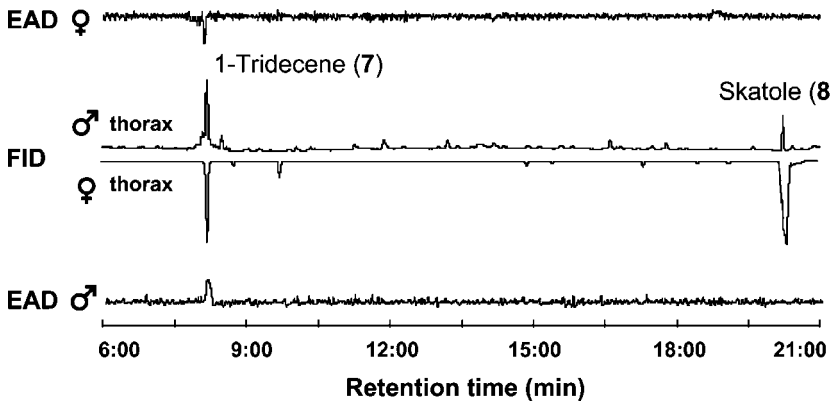

FIG. 3. GC-EAD responses of *Chrysopa oculata* to thoracic extracts of goldeneyed lacewing adult males and females.

sensitive to (1*R*,2*S*,5*R*,8*R*)-iridodial with its EAD-response threshold being between 0.1 and 1 pg (or lower), which is 10–100 times lower than thresholds for nonanal and nonanoic acid (Figure 4A), and ca. 1000–10,000 times lower than others (Figure 4A and B). Male antennae of another *Chrysopa* species, *Co. quadripunctata* (females were not available for test), showed a similar EAD response pattern to the synthetic mixtures as that seen for *Co. oculata* (Figure 5B).

Antennae of *Chrysoperla rufilabris* showed similar EAD responses to most of the compounds in the synthetic mixture (only 100-ng dose tested), but were unresponsive to (1*R*,2*S*,5*R*,8*R*)-iridodial (**1**) and its (8*S*)-stereoisomer (**2**) (Figure 5A). Volatile compounds were not detected in abdominal cuticular extracts of either sex by GC or EAD in *Cl. rufilabris* (not shown). Thoracic extracts of *Cl. rufilabris* indicated the presence of one main component (**12**), which elicited EAD-responses (Figure 6). GC-MS analyses of the thoracic extracts showed that the spectrum of the main compound contained ions characteristic of a tridecene (M^+ at m/z 182), the 4–5 double bond position was determined by DMDS derivation followed by GC-MS analysis, and GC coinjection with standards established the structure of this natural product as (*Z*)-4-tridecene. The mass spectrum of the minor component eluting within 1 min after **12** indicated a molecular weight of 180, suggesting a C_{13}-diene structure. Further characterization of this compound was not pursued because this compound did not stimulate antennae in GC-EAD analyses.

Scanning Electron Microscopy. Elliptical epidermal glands (ca. 12 × 7.5 μm with a central slit) occur in great numbers on the 3rd–8th abdominal sternites of *Co. oculata* males (Figure 7A). These glands do not occur on the 1st, 2nd, or

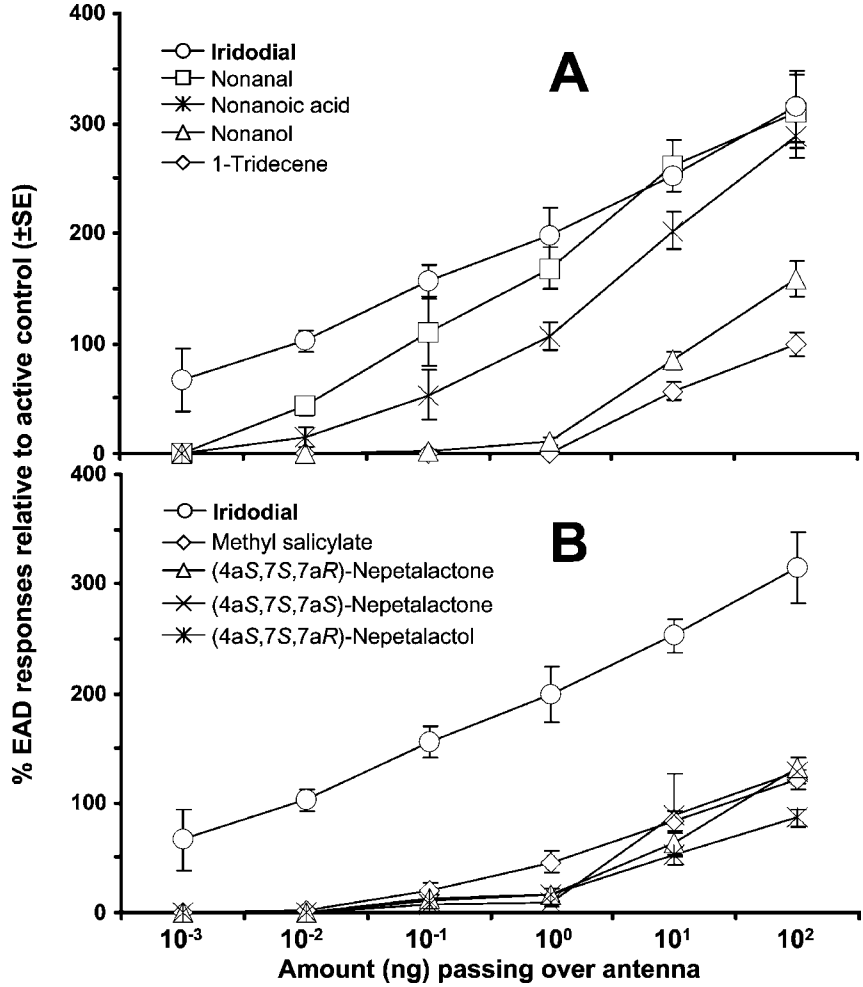

FIG. 4. GC-EAD dose-responses of *Chrysopa oculata* to synthetic mixtures ranging from 1 pg to 100 ng per test compound. (A) Comparison of (1*R*,2*S*,5*R*,8*R*)-iridodial with other *Co. oculata*- produced compounds; (B) comparison of (1*R*,2*S*,5*R*,8*R*)-iridodial with prey- or plant-produced compounds. For each dosage and compound, 4–17 GC-EAD runs were conducted. Pooled data (both male and female antennae) were used due to the lack of differences in EAD responses between sexes. The responses were normalized as the response (%) relative to an active control (100 ng of (*E*)-2-hexenyl butyrate; Mean EAD = 0.2 mV).

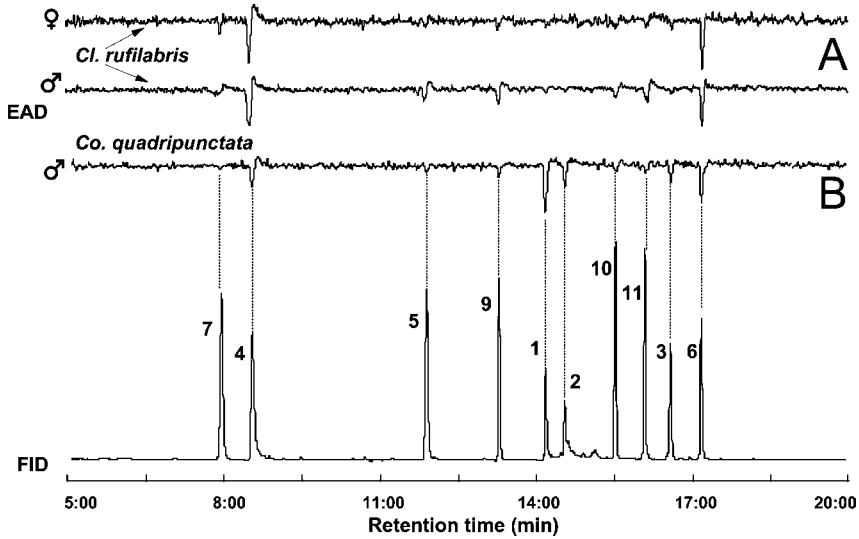

FIG. 5. GC-EAD responses of *Chrysoperla rufilabris* male and female (A), and *Chrysopa quadripunctata* male (B) to a synthetic standards (100 ng/μl each; numbers refer to structures in Figure 1).

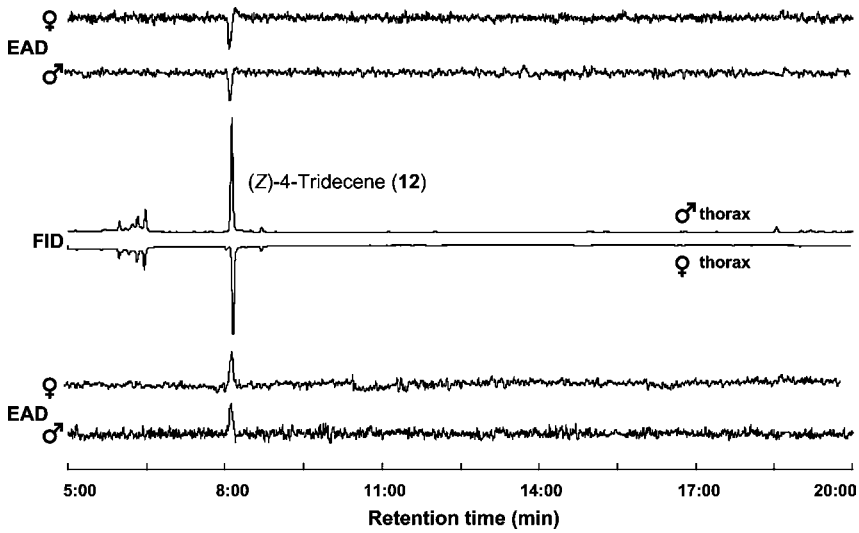

FIG. 6. GC-EAD responses of *Chrysoperla rufilabris* to thoracic extracts of conspecific males and females.

9th abdominal sternites of males, and are totally absent in *Co. oculata* females (Figure 7B). Sternites 3–8 were estimated to have about 800, 2100, 2500, 2500, 2300, and 1500 of these epidermal glands, respectively. The male-specific gland system evident in *Co. oculata* is totally absent in both males and females of *Cl. rufilabris* (Figure 7C and D, respectively). No SEMs of *Co. quadripunctata* adults were prepared.

Preliminary Field Trapping Experiment. A total of eight *Co. oculata* males were captured in clear plastic traps baited with spined soldier bug pheromone plus (4a*S*,7*S*,7a*R*)-nepetalactone (*Z*,*E*-nepetalactone) from May 30 through June 5, 2003. No *Co. oculata* females were captured, nor were any *Co. oculata* adults caught in unbaited control traps or traps baited with other treatments.

Field Trapping Experiments for Co. oculata. In experiment 1, a total of 57 *Co. oculata* males were caught in five sets of traps. Traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial or (4a*S*,7*S*,7a*R*)-nepetalactol [with 5% of (1*R*,2*S*,5*R*,8*R*)-iridodial as impurity] caught significantly more males than did blank control traps, whereas the mixture of the three male-produced C_9-compounds (**4–6**) and (4a*S*,7*S*,7a*R*)-nepetalactone (**10**) alone were each inactive (Figure 8). (1*R*,2*S*,5*R*,8*R*)-Iridodial was 4 times more attractive than (4a*S*,7*S*,7a*R*)-nepetalactol. No females were captured in the entire test.

In experiment 2, 690 *Co. oculata* males were caught. The individual C_9-compounds (**4–6**) did not attract any lacewings, and the binary and ternary combinations of these compounds were also inactive (Figure 9). Traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial alone or (1*R*,2*S*,5*R*,8*R*)-iridodial plus the three C_9-compounds in all possible combinations caught significant numbers of *Co. oculata* males. However, addition of the C_9-compounds to (1*R*,2*S*,5*R*,8*R*)-iridodial significantly reduced the trap catches (Figure 9). No females were caught in any traps in this experiment, although four eggs were found on the outside of one trap baited with (1*R*,2*S*,5*R*,8*R*)-iridodial, and 2–3 females were observed landing on the plants close to the (1*R*,2*S*,5*R*,8*R*)-iridodial baited traps. In addition, four males of *Co. quadripunctata* were captured in traps having lures containing (1*R*,2*S*,5*R*,8*R*)-iridodial.

In experiment 3, 454 males of *Co. oculata* were captured. (1*R*,2*S*,5*R*,8*R*)-Iridodial alone was again attractive (Figure 10). Traps baited with the C_9-blend (loaded on one dispenser), 1-tridecene, or methyl salicylate caught no lacewings, whereas three lacewings were caught in traps baited with (4a*S*,7*S*,7a*R*)-nepetalactone. Both 1-tridecene and the C_9-blend showed significant inhibitory effects on attraction of male lacewings to (1*R*,2*S*,5*R*,8*R*)-iridodial, with inhibitory effect of 1-tridecene being stronger than that of the C_9-blend. Combining (1*R*,2*S*,5*R*,8*R*)-iridodial and methyl salicylate resulted in a significant increase in the number of *Co. oculata* males that were caught relative to traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial alone (Figure 10). Again, no females were caught in the entire experiment.

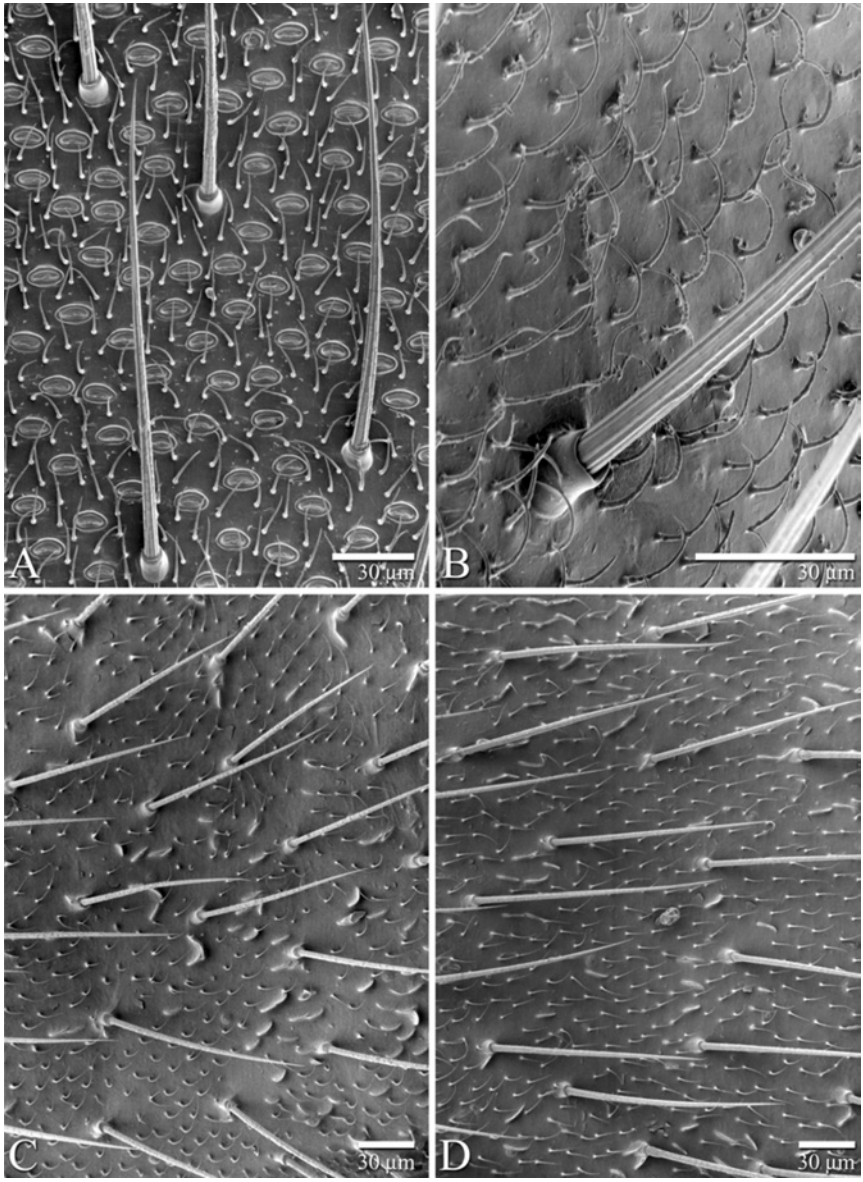

FIG. 7. SEM images of the 5th abdominal sternite of *Chrysopa oculata* and *Chrysoperla rufilabris*: *Chrysopa oculata* male (A) and female (B); *Chrysoperla rufilabris* male (C) and female (D).

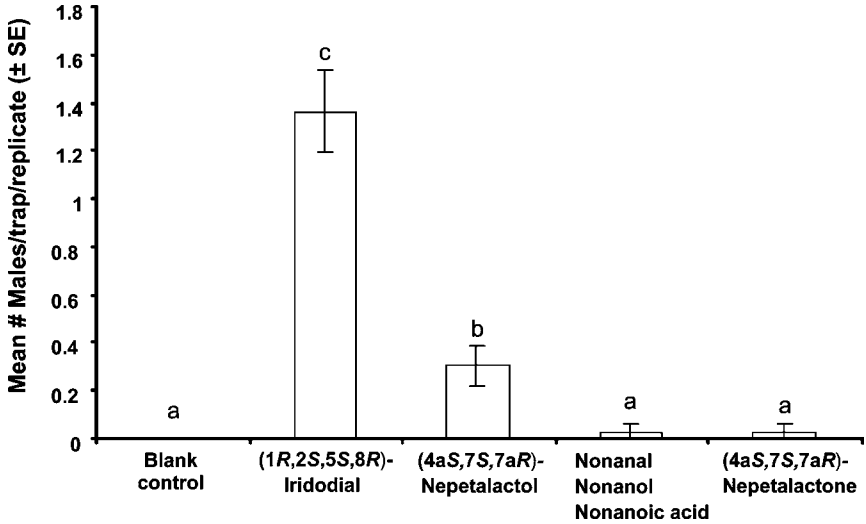

FIG. 8. Captures of male *Chrysopa oculata* in traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial, the C_9-blend, (4a*S*,7*S*,7a*R*)-nepetalactone, or (4a*S*,7*S*,7a*R*)-nepetalactol ($N = 33$, $\Sigma = 57$). Means followed by the same letter are not significantly different ($P > 0.05$), Kruskal-Wallis ANOVA on ranks, followed by Student-Newman-Kuels all pairwise comparison.

(1*R*,2*S*,5*R*,8*R*)-Iridodial, the C_9-blend, and other compounds eliciting responses from *Cl. rufilabris* antennae [(4a*S*,7*S*,7a*R*)-nepetalactone, (4a*S*,7*S*,7a*R*)-nepetalactol, and methyl salicylate] were also tested during August and September 2003, in a residential area where *Cl. rufilabris* was abundant. Over a 2-wk period using two sets of traps, 1 male and 1 female *Cl. rufilabris* were caught in the traps baited with methyl salicylate, whereas none were caught in traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial or the other compounds.

DISCUSSION

Our results with the goldeneyed lacewing, *Chrysopa oculata*, clearly indicate that (1R^*,2S^*,5R^*,8R^*)-iridodial is a male-produced pheromone that attracts conspecific males. The pheromone is probably produced in elliptical glands abundantly distributed on the 3rd–8th abdominal sternites of males. Another lacewing common in our region, *Chrysoperla rufilabris*, completely lacked the pheromone system found in the goldeneyed lacewing.

This is the first pheromone identified for lacewings, a discovery supporting speculation by Hooper et al. (2002) that attraction of male lacewings to nepetalactone and nepetalactol components of aphid sex pheromones, might be

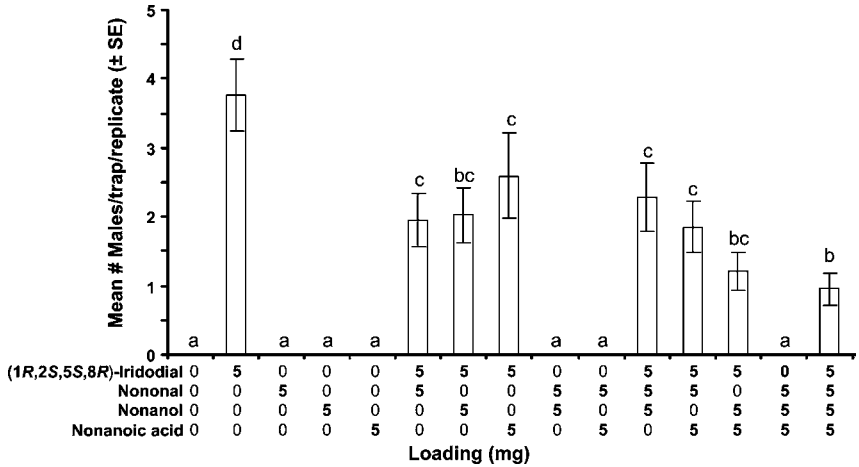

FIG. 9. Captures of male *Chrysopa oculata* in traps baited with four individual male-specific compounds, (1*R*,2*S*,5*R*,8*R*)-iridodial, nonanal, nonanol, and nonanoic acid, and all possible binary, ternary, and quaternary combinations ($N = 39$, $\Sigma = 690$). Means followed by the same letter are not significantly different ($P > 0.05$), Kruskal-Wallis ANOVA on ranks, followed by Student-Newman-Kuels all pairwise comparison.

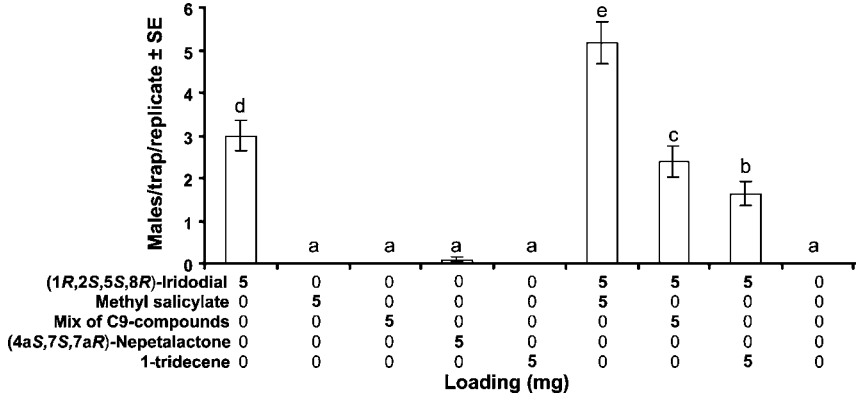

FIG. 10. Captures of male *Chrysopa oculata* in traps baited with (1*R*,2*S*,5*R*,8*R*)-iridodial alone, the C_9-blend, (4a*S*,7*S*,7a*R*)-nepetalactone, or methyl salicylate, and with some combinations of these compounds ($N = 36$, $\Sigma = 454$). Means followed by the same letter are not significantly different ($P > 0.05$), Kruskal-Wallis ANOVA on ranks, followed by Student-Newman-Kuels all pairwise comparison.

incidental to the fact that they prey upon aphids. Isomers of iridodial are unavoidable impurities in nepetalactol (Chauhan et al., 2004), the aphid pheromone component most attractive to *Chrysopa* spp. (Boo et al., 1999). In our preliminary testing, we found that *Co. oculata* males [(probably matured from overwintered prepupae (Canard and Principi, 1984)] were attracted in late spring to traps containing 15 mg of (4a*S*,7*S*,7a*R*)-nepetalactone (*Z*,*E*-nepetalactone) isolated chromatographically from catnip oil, similar to an earlier report (Boo et al., 1998). However, the tests conducted later in the season using a 5-mg dose of (4a*S*,7*S*,7a*R*)-nepetalactone isolated chemically from catnip oil by a different procedure attracted fewer lacewings than did traps baited with 5 mg of (4a*S*,7*S*,7a*R*)-nepetalactol.

Iridodials have been identified from many other natural sources such as ants (Attygalle and Morgan, 1984; Nascimento et al., 1998), especially the *Iridomyrmex* spp. (e.g., Cox et al., 1989), as well as rove beetles (Staphylinidae) (Huth and Dettner, 1990), and a stick insect (Phasmatodea: Phasmatidae) (Smith et al., 1979). In all these cases, iridodials serve as defensive compounds (Attygalle and Morgan, 1984). Iridodial is even claimed to be useful in the prevention of human cancer (Anonymous, 2002), although we were unable to detect any iridodials in the commercial product (Iridodial, Global Healing Center, Houston, TX) (unpublished data).

Whereas ours is the first report of a pheromone for lacewings, chrysopids have long been known to communicate acoustically (Henry, 1982). Chrysopids produce low frequency, substrate-borne vibrations that guide the sexes to find one another on a plant (Henry, 1982). Comparative acoustic studies of *Chrysoperla* vs. *Chrysopa* species indicate that *Chrysoperla* spp. [*Cl. rufilabris*, *Cl. carnea* (Stephens) and *Cl. downesi* (Smith)] are more dependent on acoustic signals for mating success than are *Chrysopa* spp. (*Co. oculata* and *Co. chi* Fitch) (Henry, 1979, 1980a,b,c). Thus, species of *Chrysoperla* rely on acoustic communication with no obvious role for pheromones, whereas *Chrysopa* species communicate with pheromones and, to a lesser extent than *Chrysoperla*, with acoustic signals. Adults of *Chrysopa* (*sensu stricto*) are predacious, whereas *Chrysoperla* adults are phytophagous (Principi and Canard, 1984), suggesting that predation in the adult stage somehow favors chemical communication or selects against communication by substrate vibration. The work of Castellanos (2003; Castellanos and Barbosa, 2004) provides an example of how silence is favored in predatory insects: the spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), is a more successful predator of certain caterpillars than are *Polistes* paper wasps, in large part because the bug is not as noisy while hunting as are the wasps.

Surprisingly, no females of *Co. oculata* were caught during any of our experiments, even though antennae from females were as sensitive to (1*R*,2*S*,5*R*,8*R*)-iridodial and other male-produced compounds as were antennae

of males. However, four lacewing eggs were laid on one trap baited with (1*R*,2*S*, 5*R*,8*R*)-iridodial, and three females were observed on plants nearby (1*R*,2*S*, 5*R*,8*R*)-iridodial baited traps. Therefore, it is possible that female lacewings are also attracted to (1*R*,2*S*,5*R*,8*R*)-iridodial, but do not approach close enough to be trapped. Once a female is in the vicinity of a pheromone-calling male, it is conceivable that she produces substrate vibrations that cause the male to approach her. Indeed, in phytophagous bugs such as the southern green stink bug, *Nezara viridula* (L.) (Pentatomidae), long-range attraction to male-produced pheromones probably brings both sexes together on the same plant (Aldrich, 1995), and then substrate-borne vibrations produced by the female stimulate males to walk to the female (Çokl et al., 1999; Çokl and Virant-Doberley, 2003).

In addition to (1*R**,2*S**,5*R**,8*R**)-iridodial, the three antennal-stimulatory active C_9-compounds (nonanal, nonanol, and nonanoic acid) were also found in abdominal cuticular extracts of males. These C_9-compounds alone or in blends were unattractive to *Co. oculata*, and reduced attraction of the males to (1*R*,2*S*,5*R*,8*R*)-iridodial. Individual variation in the relative amounts of (1*R**,2*S**,5*R**,8*R**)-iridodial (coefficient of variation = 112%) is much greater than that for the C_9-compounds (CV = 20–56%). Such a large individual variation for iridodial might indicate that the sampled *Co. oculata* males were physiologically different, and that the C_9-compounds may play a role independent from that of iridodial. As one might expect for insect pheromones, the EAD-response threshold of *Co. oculata* to (1*R*,2*S*,5*R*,8*R*)-iridodial (0.1–1 pg) was 10–100 times lower than that for nonanal and nonanoic acid, and 1000–10,000 times lower than that observed for nonanol, 1-tridecene, and other compounds tested. *Chrysopa quadripunctata* also showed an EAD-response pattern similar to that of *Co. oculata*, but too few insects were available to pursue pheromone identification. *Chrysoperla rufilabris* showed no EAD response to (1*R*,2*S*,5*R*,8*R*)-iridodial, which correlates with lack of behavioral responses to this compound by *Cl. rufilabris* adults. Interestingly, however, *Cl. rufilabris* antennae were about as sensitive to the C_9-compounds as were *Co. oculata* antennae, which supports independent roles for iridodial and the C_9-compounds. Our analysis of the abdominal tip extract failed to detect EAD-active compounds, even though *Co. oculata* males possess eversible vesicles near the tips of their abdomens that are suspected to be pheromone glands (Hwang and Bickley, 1961).

Elliptical epidermal glands occur on the 3rd–8th abdominal segments in several European *Chrysopa* species (Principi, 1949, 1954a,b). In *Co. septempunctata* Wesmael (Principi, 1949) and *Co. viridana* Schneider (Principi, 1954a), the glands are distributed on both tergites and sternites, whereas in *Co. perla* (L.), these glands exist only on the sternites (Principi, 1954b), a distribution as in *Co. oculata*. In other lacewing species, such as *Pseudomallada flavifrons* (Brauer) and *P. ventralis* (Curtis), similar integumental structures occur on the male thoracic

tergites, whereas in *Cl. carnea*, *Nineta flava* (Scopoli), and *N. vittata* (Wesmael) (Principi, 1954b), as well as in *Cl. rufilabris* (this paper), elliptical glands are absent. On the basis of our SEM observations of *Co. oculata*, we estimate that a male has almost 12,000 elliptical glands (ca. 2,000/mm^2), a glandular abundance equivalent to that of *Co. septempunctata* (Principi, 1949). The fact that the distribution of elliptical glands in *Co. oculata* males coincides with extraction of the pheromone from the 3rd–8th abdominal sternites strongly implicates these glands as the source of the pheromone. We suspect that *Co. quadripunctata*, and other chrysopid species with epidermal glands as found in *Co. oculata* males, possess pheromone systems similar to that of the goldeneyed lacewing. In fact, the attraction of males of various chrysopid species to dihydronepetalactols (neomatatabiol and isoneomatatabiol) from the Japanese plant, *Actinidia polygama* Miq. (Actinidiaceae), may be due to the production of these or related compounds from male chrysopids themselves (Hyeon et al., 1968; Sakan et al., 1970; Hooper et al., 2002).

Chemical defense in the goldeneyed lacewing (Blum et al., 1973) and other chrysopids (Zhu et al., 2000) is thought to be accomplished by secretion from a pair of thoracic glands. Our analyses of *Co. oculata* thoracic extracts confirmed the results of Blum et al. (1973) that 1-tridecene and skatole are produced by both sexes. Surprisingly, skatole, a compound strongly organoleptic to humans and other animals, elicited no EAD response from *Co. oculata*, whereas 1-tridecene elicited a strong response from antennae of both sexes of this species. In our study, addition of 1-tridecene to (1*R*,2*S*,5*R*,8*R*)-iridodial-baited traps significantly reduced the numbers of *Co. oculata* males captured. This antagonistic effect indicates that 1-tridecene might also function as an alarm or antiaggregation pheromone. In contrast to *Co. oculata*, no skatole was detected from thoracic extracts of *Cl. rufilabris*, but a significant EAD response was elicited from antennae of both sexes to the major component (common in both males and females) identified as (*Z*)-4-tridecene. Similarly, in *Cl. carnea*, (*Z*)-4-tridecene was identified as the main thoracic gland component, and stimulated antennae, but skatole was absent (Zhu et al., 2000).

Whereas attraction of chrysopids to nepetalactone, nepetalactol, and dihydronepetalactols may not be directly related to the occurrence of these compounds in plants, at least some herbivore-induced plant volatiles appear to be ecologically relevant signals guiding lacewings to prey-infested plants (Han and Chen, 2002; James, 2003). Methyl salicylate, a volatile induced by phloem-feeding whiteflies and aphids (Walling, 2000), significantly attracted males and females of *Co. nigricornis* in the field (James, 2003). Our tests of methyl salicylate at a dose of 5 mg/lure, a much lower dose than that used by James (2003), failed to attract *Co. oculata*. However, methyl salicylate significantly increased attraction of *Co. oculata* males to (1*R*,2*S*,5*R*,8*R*)-iridodial. This synergism between iridodial (a pheromone) and methyl salicylate (a synomone in this context) supports the

concept that coincidence of chemical signals from different trophic levels together constitutes a more powerful attractant blend than a monotrophic signal (Zhang and Schlyter, 2003). (1*R*,2*S*,5*R*,8*R*)-Iridodial alone or its combination with herbivore-induced plant volatiles (e.g. methyl salicylate) and/or prey-produced kairomones may be of practical utility in manipulating natural or artificially augmented populations of lacewings for enhanced biological control.

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